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NUTRITION REVIEWS

VOL. 16 AUGUST 1958 No. 8

NUTRITION AND CHRONIC DISEASE CONTROL

Improved nutrition among the world's poorly nourished people will do more to raise health and economic standards than any other single factor. The food industries can do more than most others to strengthen our country's position in the struggle for world peace and prosperity, because their role is to serve mankind's needs for the right food and enough of it. The Russian Sputnik has indeed captured the imagination of space enthusiasts the world over. But relief from hunger and disease is the sharp, ever-present need of the world's billion under-privileged people. In the last analysis, the struggle for their minds and hearts will be won by the society that does the most to meet the human needs for food and health.

In our own country, we have made tremendous strides in the improvement of health and nutrition throughout this century, especially in the past two decades. Since the advent of antibiotics, the emphasis in health education of the public has been on the continuing rapid introduction of new pharmaceutical products, the new vaccines and the "miracle drugs". We have not, I think, given sufficient emphasis to the wonders performed by the food industry in the same period.

Advances in production, processing, preservation, transportation and marketing of foods in the past 20 years have been as dramatic, as much a "miracle", as pharmaceutical progress. The contribution of the food industries to public health, indeed, is so large that we may never be able to measure its results. The average housewife's food dollar, even in a period of inflation, buys much more in high-quality nutrients than it was possible for her mother to buy 25 or 30 years ago.

The continuing trends of a healthy economy (growing population, high employment, and rising family income) have, of course, greatly increased consumer demand for foods. But the rising standard of living would not, of itself, have made such a strong impact on nutritional standards without accompanying progress in the food industries.

There is no season of the year when plentiful supplies of fresh fruits and vegetables in amazing variety (milk, cheese, eggs, butter and margarine, meats, poultry, fish and seafood) are not available to the smallest community in the land, as are the countless other items on the grocery list that makes us a well-fed nation. Nowadays, visitors from overseas find our suburban super-markets more fascinating, more typical of the American scene than our sky-scrapers.

There is a specially bright spot in this picture of plenty; that is, infant nutrition. Here, long-established cooperation between nutrition scientists, pediatricians, and industry has saved the lives of millions of infants, and has actually revolutionized the feeding of babies and young children. Quite apart from the mass production of milk formulas and the addition of a wide range of foods to the infant's diet, I am sure our babies are better nourished just because their mothers have been relieved of the strain and tedium of preparing their foods "from scratch".

American children are considerably taller and heavier than they were 20 or 30 years ago. This gain in height and weight was observed some years ago in children born in the 1920's. Doctors Hundley and Mickelson of the Public Health Service have found this trend to be continued, at least as late as 1949, in their recent comparison of the height and weight records of seven to nine-year-olds in Philadelphia.

In sum, the high nutritional level of the

American people is due to the fortunate interplay of medical science, food technology, education and the national economy. Improvement of the average diet has built stronger Americans, saved lives, and virtually eliminated such once-prevalent deficiency diseases as pellagra, rickets, and infantile scurvy. It has played an important part, along with public health measures, in the decline of tuberculosis.

Many advances in medical knowledge during the past decade have been made possible by the very rapid growth of our young nutrition science. This progress has brought medicine, public health, and the food industries to a vantage point from which we can envision new roles for nutrition in an early attack on some of the most devastating chronic diseases and hereditary disorders.

Research programs of the Public Health Service reflect the increasing emphasis on biochemistry, the basic discipline of nutritional science, not only in the studies conducted at our National Institutes of Health, but also in a large proportion of the research projects supported by grants. Biochemical research is the entering wedge for the solution of many basic problems in such diverse fields as cancer, cardiovascular, neurological, mental, and infectious diseases, as well, of course, as the major metabolic disorders.

Atherosclerosis, for example (the most common form of arteriosclerosis) is the primary cause of 97 percent of all cases of coronary artery disease, and of a high percentage of mental disorders in older people. Within the past six years scientists throughout the country have been pressing forward to identify the specific biochemical and physiological factors in the causation of atherosclerosis.

In my opinion, the food industries have been very wise in their support of basic research on these problems. Much remains to be done before the exact relationships between dietary factors and atherosclerosis can be determined. We do not yet know whether the development of preventive measures will be a problem for the food industries or the pharmaceutical manufacturers. But we have good reason to believe that dietary changes will at least play a very important part in the prevention of atherosclerosis.

The National Institutes of Health is supporting and conducting a wide range of research projects related to atherosclerosis, including studies of dietary factors. One of the important research problems, for example, is to get better chemical analyses of food fats and edible oils. Our National Heart Institute is serving as a focal point for disseminating scientific information on this problem to the shortening industries and to scientists working in all types of institutions.

We in the Public Health Service are encouraged by the wide research interest in atherosclerosis. The problem involves so many highly technical skills in the basic and clinical disciplines that we need have no fear of "duplication" in increased research on atherosclerosis. In fact, the more scientific observations we can obtain, the sooner we will be able to develop and test large-scale preventive methods against this disease which impairs the arteries of millions of Americans in the prime of life.

Obesity is believed to be widespread in the American population. Yet the solution of this health problem is far more complex than a simple reduction of caloric intake. Overweight is believed to be closely related to such chronic diseases as diabetes, hypertension, gout, and some types of arthritis.

This complex problem of obesity deserves more fundamental study. Medical science needs, for instance, much more specific data on energy metabolism as base-lines for definitive studies of obesity. At our National Institutes of Health Clinical Center, scientists are attempting to establish such base-lines. They are using a special metabolic chamber where the energy expenditure of subjects can be measured, calorie by calorie, for extended periods of 24 to 48 hours.

It is known that individuals vary in their nutritional requirements, and the individual himself varies many times during his life in the nutrient economy of his body. But we

need to know a great deal more about the peculiar nutritional needs of children at different stages of their growth and development, of pregnant women, nursing mothers, and older people. We need to know about the effects of acute illness and of certain therapeutic drugs on individual nutritional status. And we need to know to what extent the development of chronic diseases may be influenced by inadequate nutrition of individuals in such periods of stress.

Medical research is just beginning to clarify the many intricate relationships between human nutrition and chronic diseases. But there is a steady flow of fresh scientific observations which are of immediate value to practicing physicians in the management of chronic disease patients.

Studies at the National Institutes of Health are throwing light on certain dietary problems of rheumatoid arthritis patients who are being treated with synthetic steroids. Our studies are concerned with the inflammatory and metabolic effects of these drugs. It has been found, for example, that the synthetics tend to increase the excretion of nitrogen, in the course of an increased conversion of protein to sugar, resulting from these steroids. As a result, a diet fairly high in proteins is indicated for patients on these drugs. Another factor under study is the extent to which the synthetics cause sodium retention, and hence require the control of mineral intake in relation to steroid therapy. Some of the synthetics cause gastric ulceration, so that their use calls for vigorous anti-ulcer measures.

The nutritional problems of old age are sufficiently outstanding to command a great deal more attention than they are getting, both in medical research and in the application of available knowledge. The Public Health Service is conducting and supporting a considerable number of studies on the processes of aging, in addition to our large research programs on the chronic diseases. Among the studies on aging, I would like to mention the current interest of the National Institute of Arthritis and Metabolic Diseases in calcium metabolism. Laboratory and clinical investigations are underway to determine the level of calcium intake required for the maintenance of healthy bone structure as the individual ages. These basic studies are an outgrowth of our interest in osteoporosis, a condition that results in serious disability in many older people. The purpose is to learn whether the level of calcium intake plays an important role in this fundamental bone defect.

In some communities, one or more agencies are offering the services of trained dietitians or nutritionists to advise older people on their food habits. Luncheon clubs at recreational centers for the elderly assure at least one well-balanced daily meal for those who attend, as well as better information on the relation of good diet to health. Although there are no specific data to show that these services have had any effect on the health or nutritional status of the individuals, the idea is worth trying and the techniques of counseling and follow-up can be improved.

In cases where physicians desire to place elderly patients on therapeutic dietary regimens, however, the services of nutritionists would be invaluable. During their medical education, most physicians have learned to rely on the dietetics staff of the hospital for the application of dietary therapy. In practice, they seldom have access to such supplementary services for ambulatory or home-care patients. There is a great challenge to our local health agencies to furnish such services.

Improved nutrition of very old people probably will not have much influence in the prevention of chronic disease. There will be a high incidence among them of irreversible damages due to long-continued malnutrition. The preventive values of nutrition in chronic disease control must begin at the opposite end of life.

Despite advances in the feeding of infants and children, evidence is rapidly accumulating that inadequate nutrition in childhood may be an important factor in chronic disease of later years. Protein deficiency, as,

for example, in the childhood disease kwashiorkor, is associated with degenerative diseases of the liver in later life. The importance of maintaining a good diet in the gaining period is suggested by the work of Best and his co-workers on dietary choline deficiency. When weanling rats were placed on a choline-deficient diet for one week and then placed on a good diet for the rest of the time, the rats showed signs of high blood pressure in adulthood.

Even more challenging are the new facts emerging to show that nutrition of mothers and infants in the entire prenatal period has a direct bearing on certain serious congenital or hereditary diseases. Some types of mental deficiency may be due to hypothyroidism which may be corrected by dietary or hormonal therapy, or a combination of both. Two types of hereditary mental deficiency have been linked very recently with specific metabolic disorders which may be corrected by dietary measures.

For example, galactosemia has long been recognized as an hereditary inability to metabolize a specific component of milk sugar, galactose. The early manifestations are observable shortly after birth, but it has been extremely difficult to differentiate such symptoms as diarrhea, jaundice, and enlargement of the liver in infants. Deaths, therefore, have been common in early infancy and children who survive have been affected by metabolic illnesses and mental retardation the rest of their lives.

Within the past two years, Dr. Herman Kalckar and his associates at our National Institute of Arthritis and Metabolic Diseases have identified the specific biochemical defect in galactosemia as a deficiency in an enzyme, galactose transferase. On the basis of this finding, the same research workers have developed a safe and relatively simple diagnostic test for galactosemia, applicable in infants and older children. There is good reason to believe that many cases have been unrecognized in the past. It is now possible to establish a definitive diagnosis of the disease in earliest infancy, and hence to prevent its devastating effects through milk-free diets. Since the mother's milk is as dangerous to the galactosemic infant as any other milk formula, there is a challenge here to develop milk-free diets for the newborn and older babies afflicted with this metabolic deficiency.

The advancing front of medical research is revealing more and more fundamental knowledge of man's biochemical functioning. These findings are emerging from many laboratories, and from many studies focused upon innumerable specific problems. We are beginning to see new principles of pathogenesis emerge from the combined contributions of genetics, biochemistry, biophysics and other basic disciplines. In many instances, the application of these new principles to the study of chronic diseases and hereditary disorders will lead to applied research and development of effective preventive measures. With the continued support of industry, government, and voluntary institutions, we can be sure that nutritional science will make outstanding contributions to the future prevention and control of chronic disease.

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REDUCING OBESE SUBJECTS

A variety of methods have been proposed for weight reduction programs. Generally these involve either a restriction in food intake or a drastic change in the type of diet used. Few of these programs have included physical activity as a part of the reducing

regimen. Exercise has been considered of minor significance as a means of increasing the magnitude of the calorie deficit.

A suggestion that exercise may be an effective adjunct to a weight reduction program has been presented by J. A. Strong, R. Passmore and F. J. Ritchie (*Brit. J. Nutrition* **12,** *105 (1958)*). These investigators in Edinburgh hospitalized seven obese adults whose weights ranged from 238 to 361 pounds. None of these individuals, apart from their obesity, had physical disability. They were put on a 400 calorie diet which provided about 40 g. carbohydrate, 25 g. protein, and 15 g. fat. This diet consisted of such things as small amounts of milk, fruit, lean meat, vegetable, and low-calorie rolls. Each individual walked out-of-doors for three to four hours a day. On rainy days each subject walked in the hospital corridors. Each one kept a diary of the specific things he or she did during the day, and the exact time involved therein. The diary was used in calculating the total energy expenditure. This was supplemented by tests of the actual energy required for each of these activities. The Kofranyi-Michaelis respirometer was used for measuring the rate of gaseous exchange and for collecting samples of expired air which were analyzed for oxygen (Passmore, Strong and Ritchie, *Ibid.* **12,** *113 (1958)*). The 24-hour energy output was calculated from the above data.

On this basis, the average energy expenditure for the different subjects ranged from 2850 to 3590 calories per day. Data are given for only one subject on the energy expended throughout the day. This shows that the three to four hour walk accounted for approximately 30 per cent of the total energy output. The caloric intake ranged from 370 to 550 calories. The mean daily deficit ranged from 2450 to 3100 calories. Over the 42 days of the reducing regimen, the weekly weight loss ranged from 4.7 to 6.0 pounds.

During the study, the subjects remained physically well and had only a few minor respiratory infections and orthopedic complaints (Strong, Passmore and Ritchie, *Brit. J. Nutrition* **12,** *105 (1958)*). After they became accustomed to the exercise, the subjects were amazed at the ease with which they were able to accomplish it.

The investigators were surprised that the subjects complained very little of hunger. This is truly surprising, since the subjects were in a sense "semi-starved", and one of the characteristics of semi-starvation is the constant, gnawing hunger pangs which make it difficult for the individual to think of anything other than food (A. Keys, *et al., The Biology of Human Starvation p. 833, Minnesota University Press (1950)*). The investigators suggest that the sensation of hunger "fades when the negative caloric balance is high and the subject approaches a fasting condition."

Four of the patients never developed more than a slight ketosis as shown by urinary tests. Three of them developed moderate ketosis, but this did not appear to affect their health during the weight-reduction regimen. The ketosis was probably associated with the large amount of body fat being metabolized. Respiratory quotients, taken both at rest and when walking, were usually below 0.76. These figures suggest that more than 90 per cent of the energy expended by the subjects came from the combustion of body fat. The mild ketosis in these subjects suggests that they were able to metabolize body fat more efficiently than normal individuals. The explanation for this difference is not apparent.

It was suggested that admission of the patient to the hospital instituted a drastic change in his mode of living. This offered a means of instituting the reducing regimen on a more acceptable basis. The hospital admission was also reported to have impressed the patient with the fact that obesity is a serious disease. The investigators recognized that the hospital admission might give the subject the impression that upon discharge he was cured. With this might go an abandonment of the reducing program. That this

actually happened with some of the patients is suggested by the statement that two went off the regimen after a few months. Since each subject still weighed more than 200 pounds at the end of the study, none of them could afford to abandon the program.

The study suggests that exercise can be a valuable adjunct in a weight reduction program and can make a considerable contribution to the negative caloric balance so highly desirable in this condition. In order to establish this point, it will be necessary to maintain a control group on the same regimen without the exercise. The two groups should be compared with respect to weight loss, adherence to the program, its acceptance by the subjects and their maintenance of the reducing regimen after the study is terminated. It would be desirable to validate the contribution made by exercise to a weight reduction program since the Scottish workers stated that the loss of weight was more rapid on their program than on the orthodox regimens. The study also emphasizes the fact that extremely obese individuals can metabolize large amounts of body fat without severe or disabling ketosis.

The composition of the tissue that was lost during the weight reduction program was calculated from nitrogen balance data and the energy deficit (Passmore, Strong and Ritchie, *Brit. J. Nutrition* **12,** *113 (1958)*). It was assumed that there was no significant change in the carbohydrate content of the body. These calculations indicate that the tissue lost by the subjects contained from 73 to 83 per cent fat, 4 to 7 per cent protein, and 10 to 23 per cent water. The caloric value of the tissue lost ranged from 7000 to 8000 calories per kg. These figures are the average for the entire six weeks of the reducing regimen.

There were considerable variations in water balance for the different subjects and for the different weeks for each subject. During some periods, there was an apparent water retention even though there was a loss of body weight. The nitrogen loss during the first week ranged from 4 to 9 g. per day. During the next few weeks the magnitude of the nitrogen loss was reduced, but there was no indication that any of the subjects would establish a balance on the dietary intake of 4 g. of nitrogen per day. The data suggested that in these obese subjects at least 6 to 7 g. of nitrogen per day would be required for equilibrium on an intake of approximately 400 calories.

The data presented in this report imply very strongly that the tissue lost as a result of the weight reduction program was largely fatty tissue. Although there are suggestions that the metabolism of large amounts of fat enhances the loss of water by the body, the above study indicates that over a six-week period normal hydration is re-established in the obese subjects, who were reduced as a result of the metabolism of their body fat.

VITAMIN B_{12} METABOLISM IN LIVER DISEASE

The liver is the major storage organ for vitamin B_{12} and consequently it would not be surprising to find alterations in B_{12} metabolism in liver disease. M. Rachmilewitz, J. Aronovitch and N. Grossowicz (*J. Lab. Clin. Med.* **48,** *339 (1956)*) found that the concentration of serum B_{12} was considerably elevated in patients with acute viral hepatitis and other types of hepatic disease. Serum B_{12} concentrations in normal subjects averaged 340 micromicrograms per milliliter, while in patients with acute viral hepatitis the values ranged from 800 to 6000 micromicrograms per milliliter.

The serum B_{12} in patients with liver disease was mostly in the bound form and it was considered that this elevation in serum B_{12} level could result from an increase in the serum B_{12} binding capacity. To test this point, *in vitro* experiments were conducted. Serum from normal individuals and from patients with liver disease was incu-

bated with crystalline B_{12} for 24 hours. The amount of this B_{12} which had been bound was then determined. Bound B_{12} is available to the test microorganism only after the sample is heated. Serum from patients with hepatic disease did not show any appreciable increase in B_{12} binding capacity. This indicates that the elevated serum B_{12} concentration which accompanies liver disease is not the result of an increase in the B_{12} binding capacity of the serum.

Further studies on vitamin B_{12} metabolism in liver disease have been reported by H. Baker, I. Pasher, H. P. Dolger and H. Sobotka (*Clin. Chem.* **2,** *328 (1956)*). These workers studied the urinary excretion of vitamin B_{12} following an intramuscular injection of the vitamin. Normal subjects excreted more than 50 per cent of a 50 microgram test dose in an eight hour period. In contrast, patients with a variety of liver diseases excreted less than 20 per cent of the test dose under the same conditions.

These experiments have been continued in a more recent study (H. Baker, G. Brill, I. Pasher and H. Sobotka, *Clin. Chem.* **4,** *27 (1958)*). Patients with a diagnosis of liver disease were injected intramuscularly with 60 micrograms of vitamin B_{12}, and a subsequent eight hour urine sample was assayed for B_{12} by a microbiological procedure. Control subjects were patients with diseases other than liver disease. The state of liver damage was evaluated by various clinical procedures including serum bilirubin levels, blood alkaline phosphatase titers, albumin-globulin ratios of serum, and the cephalin-flocculation test.

Patients with liver disease excreted much less B_{12} under the test conditions than did the control patients. There was good correlation between the degree of liver damage, as estimated by the standard laboratory tests, and the degree of reduction in B_{12} excretion. The authors suggest that the B_{12} excretion test may be a better measure of liver damage than the standard procedures, particularly during recovery from liver damage.

The nature of this increased retention of B_{12} by patients with liver disease remains to be elucidated. The results of Rachmilewitz, *et al.* (*loc. cit.*) suggest that an increase in serum B_{12} binding capacity is not the explanation. Further study of this phenomenon may elucidate the role of the liver in B_{12} metabolism, and the B_{12} excretion test may develop into a useful laboratory procedure for evaluation of liver function.

PYRIDOXAL PHOSPHATE IN HUMAN BLOOD

Vitamin B_6 nutrition in man has been concerned with the determination of dietary intake and urinary excretion of the vitamin and its oxidation products (J. C. Rabinowitz and E. E. Snell, *Proc. Soc. Exp. Biol. Med.* **70,** *235 (1949)*).

Since it is known that vitamin B_6 functions in the conversion of tryptophan to nicotinic acid, an estimate of its functional sufficiency is based on xanthurenic acid and N-methylnicotinamide excretion following the administration of tryptophan (H. P. Sarett and G. A. Goldsmith, *J. Biol. Chem.* **177,** *461 (1949)*). Xanthurenic acid is an abnormal metabolite of tryptophan which occurs in the pyridoxine-deficient animal (*Nutrition Reviews* **14,** *183 (1956)*).

The essential nature of vitamin B_6 in the diet of infants has been demonstrated (C. J. Molony and A. H. Parmelee, *J. Am. Med. Assn.* **154,** *405 (1954)*; D. B. Coursin, *Ibid.* **154,** *406 (1954)*). An inadequate intake of the vitamin for meeting the increased requirements during pregnancy has been inferred from the studies of M. Wachstein and A. Gaudaitis (*J. Lab. Clin. Med.* **42,** *98 (1953)*).

As to the function of vitamin B_6, the coenzyme form, pyridoxal phosphate, serves as catalyst in a wide variety of biochemical

reactions of amino acids. The coenzyme is primarily an intracellular constituent of animal tissues.

A recent paper presents data on the level of the codecarboxylase, pyridoxal 5-phosphoric acid (B_6-PO_4), in human whole blood and isolated white blood cells (G. E. Boxer, M. P. Pruss and R. S. Goodhart, *J. Nutrition* **63,** *623 (1957)*). The assay method is based on the rate of decarboxylation of tyrosine by a B_6-PO_4-activated tyrosine decarboxylase.

Whole blood analyses for B_6-PO_4 in 224 adult human subjects are reported. Only 32 of these subjects contained a sufficient amount of the coenzyme to allow detection (10 millimicrograms per ml.) by the method used. The highest value observed was 37 millimicrograms. This is to be contrasted with the considerably higher average whole blood values found by the authors in a number of mammalian species (guinea pig, 32; rat, 187; rabbit, 118; cat, 110; and dog, 49 millimicrograms per ml.). In the rhesus monkey, as in adult man, the average whole blood B_6-PO_4 was also found to be below the detection limits of the method.

The whole blood of 18 children below 18 months of age averaged 32 millimicrograms per ml., with none as low as the detection limits of the analytical method.

The effect of increased dietary intakes of vitamin B_6 on whole blood B_6-PO_4 was determined in the adult subjects. Blood samples drawn two to four hours after daily oral administration of 0.5, 1.0, 2.5, 5, and 50 mg. of pyridoxine HCl yielded B_6-PO_4 values of 19, 24, 38, 44, and 42 millimicrograms per ml., respectively. Thus, the ability to convert oral vitamin B_6 into enzymatically-active B_6-PO_4 appeared to proceed efficiently with a daily intake of approximately 7 mg. of the vitamin (2 mg. from the natural diet, plus 5 mg. given in pure form). Pyridoxal, given to a smaller group of patients, led to about the same blood B_6-PO_4 levels as were observed with pyridoxine.

White blood cell values for B_6-PO_4 were determined on adults and on children 11 to 13 years of age. As with whole blood, the leukocyte values were again found to be higher in children (0.24 to 0.38 millimicrograms per million leukocytes) than in adults (0.14 to 0.15 millimicrograms per million leukocytes).

The authors point out that this decline in tissue concentration of B_6-PO_4 may represent an increasing demand for the vitamin with advancing age.

In a group of 127 Cuban children, B_6-PO_4 concentrations in leukocytes averaged 0.38 millimicrograms per million, contrasted with an average value of 0.24 for 88 New York City children. The authors point out that this may be a reflection of higher pyridoxine intake in Cuba as a result of the use of a variety of beans that has been found to be comparable to meat in pyridoxine content.

In separate experiments it was demonstrated that an increase in pyridoxine intake led to an increase in leukocyte B_6-PO_4. Ten to 20 days were required before the leukocyte response was evident, contrasted to a rise in whole blood B_6-PO_4 within three days after oral pyridoxine administration.

Exploratory data on the significance of leukocyte B_6-PO_4 content in arteriosclerotic disease was also obtained. In 36 patients with such disease there were seven cases with barely measurable leukocyte B_6-PO_4 (less than 0.05 millimicrograms per million). There was no obvious correlation between clinical findings and the leukocyte B_6-PO_4 level, although the results suggested a more detailed study would be desirable.

Pyridoxal phosphate also is believed to function as the coenzyme for the transaminases, and it is of interest that periods of six to seven weeks of oral pyridoxine supplementation were required to bring about stabilized, elevated blood transaminase levels in human subjects and in monkeys (*Nutrition Reviews* **14,** *80 (1956)*). These interesting studies provide a basis for extending our knowledge of the function of vitamin B_6 in human nutrition.

FEEDING THE NEWBORN INFANT

The feeding patterns of infants and young children in this country can be assessed by many different types of nutrition surveys. Detailed dietary histories on a small sample of the population (*Nutrition Reviews* **16,** *67 (1958)*) are at one end of the spectrum. On a much larger scale, the feeding practices of infants in hospitals throughout the country have been compiled in a survey by H. F. Meyer (*Pediatrics* **21,** *288 (1958)*). A questionaire containing 21 items was submitted, with a return of 63.9 per cent, to the nursery supervisors of the 2,981 hospitals having birth rates of 300 or more infants in 1955. In this survey, which had a geographical distribution representative of the country as a whole 2,225,000 newborn infants were included. Among these infants, approximately four-fifths were artificially fed, either wholly or in part; this survey is limited to the details of artificial feeding.

Eighty-two per cent of the reporting hospitals used a "house formula" which had remained unchanged for over 12 months in 88 per cent of the nurseries. In approximately 90 per cent of the hospitals utilizing a "house formula" it was selected by the pediatrician in charge, or by a committee of physicians. In only 8.2 per cent was it selected by the hospital superintendent or a representative of the purchasing department. In 817 hospitals (37.4 per cent) the milk products used in their "house formulas" were received free of charge from the manufacturer. Carbohydrate additives were reportedly received by 921 hospitals free of charge on a similar basis. The composition of "house formulas" showed no particular geographic variation.

The majority of reporting hospitals used only one "house formula," and this consisted of various modifications of evaporated milk in 65.8 per cent, premodified prepared formulas in 30.9 per cent, pasteurized milk in 3.1 per cent, and pasteurized skim milk in 0.2 per cent. Among those hospitals having two "house formulas" the great majority had evaporated milk and premodified prepared formulas as the two alternatives. Although the survey does not include detailed information concerning carbohydrate modifiers it is pointed out that 11.5 per cent of the hospitals used evaporated milk without added carbohydrate as the "house formula," without specifying the extent of the dilution.

Included in the questionaire, and of considerable interest in the interpretation placed on this survey, is the average age, in days, at which infants left the hospital. This varied between two and seven, or more, days, but the great majority of infants stayed in the hospital only four or five days following birth.

In commenting on the results of this survey, Meyer suggests that a survey based on practices which apparently have been successful over a long period of time in any specific hospital nursery would suggest that they were "successful" in terms of the adequate performance of the infants involved. However, it would seem that the survey more accurately represents current practices in nurseries for the newborn in our hospitals than evidence that the formulas used are nutritionally optimal. It should be taken for granted that "house formulas" which produced obvious clinical disturbances, either during the newborn period or in the weeks or months following discharge, would not long be continued; consequently it might be restated that the formulas receiving wide use in this survey were those which were well tolerated by the infant population. It would certainly be unlikely that a major assessment of their nutritional value could be accomplished during five days. One might assume that the ingestion of these formulas was compatible with appropriate neonatal readjustments in water and electroyte balance.

Since these data involve practices only during the first few days of life, which may or may not be continued after leaving the

hospital, it would not be appropriate to extrapolate the fact that 30.9 per cent of the infants in the country were receiving premodified prepared formulas as their sole source of milk. Meyer states "Apparently the feeding of about 15 per cent of infants in this survey was changed to evaporated milk at the time of discharge from the hospital."

Dr. Meyer has performed a great service in carrying out this extensive survey of feeding practices in our hospital nurseries which, as he so aptly suggests, may prove to be of considerable historical interest in the future. At a time when the practices of infant feeding are being subjected to various commercial and economic pressures, as well as to the effects of disagreement and uncertainty concerning optimal nutritional requirements, this survey may serve as a base line for evaluating future trends in the feeding of newborn infants.

WHEAT GLUTEN IN ADULT STEATORRHEA

Coeliac disease in children, known also as non-tropical sprue or idiopathic steatorrhea, has been the subject of numerous studies (*Nutrition Reviews* **11,** *199, 261* (*1953*)). This disease, which is characterized by defective fat absorption and diarrhea, eventually leads to a state of malnutrition involving vitamin, electrolyte and fat deficiencies, and retardation of growth. Treatment with ACTH or cortisone has been effective in the relief of symptoms (*Ibid.* **10,** *169* (*1952*)), but exclusion of wheat or rye from the diet has been the most satisfactory treatment. The presence of large amounts of glutamine in the gluten of wheat or rye (*Ibid.* **14,** *165* (*1956*)) has been implicated in the production of the harmful effect.

J. M. French, C. F. Hawkins and N. Smith (*Quart. J. Med.* (*New Series*) **26,** *481* (*1957*)) have reported their study of the effect of a wheat-gluten-free diet in adult idiopathic steatorrhea. In their examination of numerous case reports of adults treated for idiopathic steatorrhea by exclusion of wheat or rye, they noted that formerly, little success by this treatment had been achieved in older patients. The fact that many adults suffering from this condition had had a history of intestinal disease in childhood (T. E. H. Thaysen *Non-Tropical Sprue, London* (*1932*)), suggested to them that exclusion of wheat-gluten might be effective in treating adult patients with this type of history.

The report of French, Hawkins, and Smith comprises a series of 22 adult patients with a diagnosis of idiopathic steatorrhea. All had suffered ill health for many years, characterized by loss of weight, diminished appetite, sore mouth and tongue, flatulence and loose stools. Tetany was seen in some instances and episodes of glossitis and stomatitis in most instances, but not all. In general the only other signs of disease were those which could be attributed to malnutrition.

The clinical diagnosis of idiopathic steatorrhea, as distinct from the syndrome due to pancreatic insufficiency, was confirmed as follows: (1) Fat absorption was less than 90 per cent; (2) juice aspirated by duodenal intubation contained normal concentrations of amylase, lipase and trypsin; (3) glucose tolerance tests showed a delayed and diminished rise in blood sugar level; (4) blood examination showed anemia; (5) radiological examination of the gastrointestinal tract using barium sulphate meals revealed the absence of organic disease of the stomach or duodenum, but did show moderate to severe dilatation.

In order to achieve rigid exclusion of wheat and rye from the diets, it was necessary to prepare bread or cake made from rice, corn, soya bean or potato flour. In some cases the

diarrhea was treated with kaolin and opium derivatives. Electrolyte imbalances were corrected using sodium and potassium salts. Folic acid and vitamin B_{12} were given to correct shortages of these vitamins. Corticotrophin (ACTH) was also used in an effort to hasten recovery.

Once diagnosis had been confirmed, either from previous history or during a control period of feeding a diet containing wheat, all patients were placed on the wheat-gluten-free diet. The estimation of fecal fat, regular weighing, and frequent examination of the blood picture permitted close check on the progress of recovery. Complete recovery was characterized by the disappearance of clinical symptoms including diarrhea, glossitis, stomatitis and the return of normal appetite and energy, normal blood picture, and normal fat absorption accompanied by weight gain. Nine patients recovered entirely with exclusion of wheat gluten as the only form of treatment. Seven other patients recovered but received folic acid, cyanocobalamin and corticotrophin (ACTH) along with the wheat-gluten-free diet. In six patients that failed to respond, no differences could be observed to account for the failure, but several possible explanations were considered: (1) The trial period may not have been long enough, or the diet may not have been entirely free of gluten; (2) the patients could have been sensitive to other substances in the diet; (3) vitamins or other essential nutrients may have been deficient; (4) altered quality or quantity of bacterial flora could have influenced absorption; (5) the steatorrhea could have been secondary to some other disease of the small intestine.

This study revealed the advantage of giving a gluten-free diet to adult patients suffering from idiopathic steatorrhea, and demonstrated that a much longer feeding trial had to be used in order to be sure that wheat gluten was not the cause of the disease in older patients. The fact that not all patients diagnosed as having idiopathic steatorrhea respond to the gluten restriction regimen indicates the possible existence of other etiologic factors.

REFRACTORINESS TO INTRINSIC FACTOR

There are suggestions that intrinsic factor concentrates prepared from one animal species are not able, in all cases, to increase the absorption of vitamin B_{12} by other species of animals or by man. On the basis of the available data, it appears that there may be a species specificity in so far as the effectiveness of the intrinsic factor is concerned.

Recent work suggests further that a refractory state may develop eventually in at least some pernicious anemia patients who are treated with vitamin B_{12} and the intrinsic factor concentrates prepared from hog mucosa.

One of the earlier observations of this phenomenon was made by D. L. Mollin and G. I. M. Ross in London (*Proc. Roy. Soc. Med.* **47,** *428 (1954)*). They reported that a pernicious anemia patient, who initially showed a good hematological response to oral treatment with 15 micrograms of vitamin B_{12} and an intrinsic factor preparation from hog's stomach, developed anemia and a marked decrease in the serum vitamin B_{12} level. The suggestion was made that the anemia probably resulted from the failure of the patient to take her supplements regularly, but she "consistently denied this."

M. Schwartz, P. Lous and E. Meulengracht (*Lancet* **272,** *751 (1957)*) in Copenhagen found a number of pernicious anemia patients who behaved in a manner similar to this. One patient in their series showed an increase in hemoglobin from an initial level

of 5 g. per 100 ml. of blood to 12 g. during a period of three months when vitamin B_{12} (30 mg. per day) and a hog mucosa intrinsic factor concentrate were administered. For the subsequent six months the blood picture remained essentially the same, even though the vitamin and intrinsic factor supplement was reduced to one-third the original dose. Toward the end of the period the red cell count started to decrease, and this was followed by a decline in the hemoglobin.

Increasing the vitamin and intrinsic factor concentrates to their original levels had no influence on the course of events. The intramuscular injection of a liver extract over a period of six days (providing a total of 540 mg. of vitamin B_{12}) produced a prompt increase in the level of red cells and hemoglobin (from a level of 6 g. to 12 g. per 100 ml.). The blood picture was maintained for the subsequent 12 months when the patient received each day 3.5 g. of dried whole hog pyloric tissue, plus 1.5 g. dried liver residue used in the manufacture of liver extracts.

To validate the above observation, Schwartz and co-workers (*loc. cit.*) carried out the Schilling test on subjects given, on separate occasions, increasing amounts of dried hog pyloric mucosa. Their procedure was to give the patient 0.5 mg. of labeled vitamin B_{12} and various amounts of hog pyloric mucosa preparations as a source of the intrinsic factor orally, and two hours later to inject 1 mg. of unlabeled vitamin. The radioactivity of the subsequent 24-hour urine sample was used as a measure of the absorption of the vitamin. When the dose of dried hog pyloric mucosa was increased up to 100 mg. per day, the radioactivity of the urine samples from normal subjects increased proportionately. Further increases in the dose of intrinsic factor produced no change in the absorption of the vitamin as measured by the urinary radioactivity.

Untreated pernicious anemia patients, and those who had been on injection therapy, showed a response to the above test which was similar to that of the normal subjects. When pernicious anemia patients who had been treated orally with vitamin B_{12} and hog pyloric mucosa for a period of six months or longer were tested, some of them showed the normal response. However, six of these ten patients excreted practically no radioactivity in the urine, even when the dose of hog pyloric mucosa was increased to 400 mg. Similar results were secured when a potent intrinsic factor preparation from hog pyloric mucosa was used.

Although some of the patients showed no response in the Schilling test to high levels of hog pyloric mucosa, they did excrete normal amounts of radioactive cobalt when 100 ml. of normal human gastric juice were given together with the tagged vitamin.

Schwartz and co-workers (*loc. cit.*) suggest that the poor absorption of vitamin B_{12}, when given orally with a hog pyloric intrinsic factor preparation, is due to the development of a refractory state in the patients. They postulate that the development of the refractory state results from a "block" in the absorption mechanism. The cause of the "block" is unknown, but it is not apparent when homologous (from the same species) intrinsic factor is given to the "refractory" patients.

Partial confirmation for the above observations comes from A. Killander in Sweden. He reported in a note (*Lancet* **272,** *104 (1957)*) that, when pernicious anemia patients were maintained on a hog intrinsic factor preparation with vitamin B_{12}, the originally high serum vitamin levels gradually decreased after the first year. After that, hematological relapses became increasingly common. Increasing the oral dose of intrinsic factor produced no improvement in the blood picture.

The unique part about Killander's report (*loc. cit.*) is the observation that after a refractory state developed to hog pyloric mucosa prepared by one manufacturer, a similar preparation from another source produced a dramatic hematological response. However, after a period of six months or more, the pa-

tients developed a refractory state to the new preparation.

The above observations suggest that when some pernicious anemia patients are treated with intrinsic factor concentrates prepared from hog mucosa, they may develop a refractory state which is manifested by a poor absorption of vitamin B_{12}. Even after the refractory state has developed, normal absorption of the vitamin can be achieved when gastric juice from normal human subjects is given. There is no work which indicates how long human gastric juice may be used with efficacy by pernicious anemia patients. These findings should spur the efforts to isolate and characterize the intrinsic factor, both in hog mucosa and in human gastric juice.

CLIMATE AND CALORIC INTAKE

It is generally held that cold environmental temperatures increase the caloric requirement. Scientific documentation for this view has come from studies such as that carried out by R. E. Johnson and R. M. Kark (*Science* **105,** *378 (1947)*) who collected dietary data on American soldiers serving in many parts of the world. Food intake was estimated to increase as a linear function with decreasing environmental temperature. Such information was influential in the recommendations of the Committee on Caloric Requirements of the Food and Agricultural Organization (FAO) of the United Nations in 1950 (*Nutrition Reviews* **9,** *40 (1951)*). This report concluded that "the relationship between calorie requirements and mean annual external temperature is approximately linear and that satisfactory allowance can be made for climatic differences by a 5 per cent increase or decrease in requirements for every 10° C decrease or increase in temperature from 10° C."

A needed re-examination of this question has been conducted under more carefully defined conditions by B. E. Welch, E. R. Buskirk and P. F. Iampietro (*Metabolism* **7,** *141 (1958)*). The food intake of United States Army volunteers was observed under controlled conditions at Fort Churchill in Canada on two occasions when the mean temperature was −22.5° F and −7.3° F for the three-to four-week study periods. For comparison similar observations were conducted at Natick, Massachusetts and at Yuma, Arizona, where the mean temperatures were, respectively, 72° F and 91° F. Physical activity was limited to a daily march of nine to ten miles over level ground except that more rigorous physical activity was performed during the second study conducted in the subarctic. Caloric intake, energy expenditure, body weight, water intake and urine output were measured.

The caloric intake per man was higher in the Fort Churchill group than in the groups studied in warm climates. Because the men selected for subarctic duty were considerably heavier, recalculating the data as caloric intake in calories per kilogram of body weight almost completely eliminated the differences between the groups studied. With moderate work 47 to 49 calories per kilogram body weight were eaten. With heavy work at Fort Churchill 60 to 62 calories per kilogram were selected. In terms of experimental design, the marked differences in body weight present between the groups weakens the study.

The energy expenditure necessary to walk at 3.41 miles per hour was found to be similar when expressed in terms of body weight for the three environments. The energy expenditure of sedentary activities for the groups was comparable when the unit of comparison was weight$^{0.7}$. This exponential expression was justified on the basis of previous studies of basal oxygen requirements.

Incidental studies were also conducted on water requirements at Fort Churchill. The

results were not notably different than those reported for temperate conditions. Naturally at Yuma, Arizona, the water intake was greatly increased and urine volume was decreased.

The influence of cold exposure on caloric intake has been examined under laboratory conditions by P. F. Iampietro, D. E. Bass, and E. R. Buskirk (*Metabolism* **7,** *149 (1958)*). The climate to which the body is exposed is not directly related to ambient temperature in the field studies mentioned above but is largely controlled by the adequacy of clothing and shelter. These considerations prompted an investigation of temperature effects on caloric intake in the absence of the modifying influences of clothing and activity.

Four subjects, clad only in shorts, were exposed for two-week periods to 80° F, 60° F and 80° F. Only sedentary activities were permitted. A single blanket was used at night during the cold period. It should be pointed out that 60° F is close to the lowest temperature which can be tolerated by the exposed subject without increased activity or shivering. A supplemented formula diet was provided in unlimited amounts. Caloric intake increased almost 500 g. during the 60° F period. There was, however, a weight loss of 107 to 78 g. daily during the warm periods and no weight loss during the cold period. When the caloric equivalent of the weight loss (estimated at 3.5 calories per gram of weight) was added to the caloric intake during the warm periods, the increased caloric intake during the cold period was only about 200 calories. The estimated resting energy expenditure for the two environments was found to differ by the same amounts.

In summary, the results of these nutritional studies on young soldiers fail to indicate a major influence of ambient temperature on caloric requirements. Indirect factors, such as the increased work involved in wearing and performing duties in heavy protective clothing, are responsible for most of the increased caloric requirements of cold climates. The extra work involved in marching with heavier boots is not the least of these indirect caloric costs. In arriving at an estimate of food requirements in the subarctic, knowledge of the type of work to be performed is of more importance than climate for individuals with adequate clothing and shelter.

ARTIFICIAL FEEDING AMONG THE BAGANDA

The history of artificial feeding of infants in western civilization dates back to the onset of recorded history. Consequently, it is only among other civilizations that we are able to see the effects of the introduction of artificial feeding of infants on a population unaccustomed to this practice in the past. Under the intriguing title, "Bottle Feeding: A Problem of Modern Civilization," H. F. Welbourn (*J. Trop. Pediat.* **4,** *157 (1958)*) has discussed the incidence of bottle feeding during the past five years among the children of a relatively advanced and well educated African tribe, the Baganda, living near Kampala, Uganda.

Until recently, breast feeding was accepted by African mothers as the only means of rearing their babies. During the past few years, however, it has become a common sight in Uganda to see an African baby being fed, often by an older child, with various mixtures of milk, cereal, or tea from a bottle. Welbourn reports findings made during the previous six years at child welfare clinics in the villages near Kampala in the years 1950 and 1955. The 1955 group was subdivided into highly educated families (graduates or secondary school teachers), and other families, among which usually one of the parents was literate. The problem of twins and orphans was considered separately.

The clinic records of 223 infants in 1950

and 285 seen in 1955 were compared. The use of supplementary bottle feeds before the age of six months increased from 14 to 30 per cent. The infants completely weaned from the breast before the age of three months remained the same at 3 and 4 per cent, respectively. The use of supplementary bottle feeds before the age of one month increased from 2 to 5 per cent, and the number of infants completely weaned from the breast before the age of one month increased from 0.9 to 1.4 per cent. The average duration of breast feeding in 1950 was 11.8 months. In 1955 the average duration was 12.7 months in those not receiving supplemental feeds, and 6.6 months in those being fed from a bottle as well.

Excluding the children of highly educated families, an interesting effect was noted of the amount of education on the incidence of breast feeding. There was no increased tendency to bottle feed among the more highly educated families. Among those families in which the parents had completed secondary education the mothers were anxious to breast feed for at least six months, and the feeding regimen followed lines suggested in English books on mothercraft.

The reasons for artificial feeding in 75 children are of considerable interest. In four, the mother was ill, in five the baby was ill, eight infants were premature, and in nine cases supplementary feeds were advised at the clinic or hospital. In eight others the supplement was probably necessary, although the mother did not ask for advice. The largest group, 41, represented infants in whom the supplement was probably unnecessary and whose mothers did not ask for advice concerning feeding at the clinic.

The effects of bottle feeding were assessed in three ways. The average weight gain of the children given supplementary feeds was apparently less than the average curve for all of these children. The incidence of diarrhea and vomiting was 10 per cent among the supplemented group versus 3 per cent among those wholly breast-fed in the first six months and 11 vs. 6 per cent in the second six months of life. Acute respiratory infections were also more common in the supplemented group, 18 vs. 10 per cent in the first six months of life and 24 vs. 14 per cent in the second six months of life. The incidence of malaria and the hemoglobin concentrations were the same in both groups.

The reasons for artificial feeding in this group were largely personal and related to both a loss of confidence of the mothers in their ability to nurse their infants satisfactorily and to the "snob appeal" which seems to be associated with the use of tinned milk products, as pointed out by D. B. Jelliffe (*W. H. O. Monograph* **29,** *Geneva, Switzerland (1955)*). In this regard the availability of nursing bottles and nipples in a community in which they had not previously been available can be a very dangerous development, particularly since the type of feed used was boiled cows' milk well diluted with water, or, occasionally, tea. Dried skim milk powder was available at the child welfare clinics and was used by a few mothers; one-third of them bought imported patent baby foods. Since the latter were expensive they were thought to be very strong, and consequently were diluted much more than the instructions suggested. Welbourn points out that commercial pressures were not exerted on this population, either in terms of artificial feeding or specific products.

The relatively poor progress of the supplemented infants in terms of weight gain, gastrointestinal symptoms, and acute respiratory disease can, of course, be attributed to the relatively inadequate foods which were used and cannot be compared with artificial feeding under ideal conditions. However, none of the difficulties associated with artificial feeding, appearing in this group of children with the advent of modern civilization, existed in the recent past when their mothers knew of no way of feeding their infants other than at the breast.

THIAMINE DEFICIENCY IN INFANTS

The relative rarity of pathologic observations on thiamine deficiency in infants in the United States is represented by the limited number of published case reports in the American literature. Only 11 cases were found by R. A. Davis and A. Wolf (*Pediatrics* **21,** *409* (*1958*)). All of these reported patients had recovered from their clinical disease. One case of Wernicke's encephalopathy and four cases of disease of the central nervous system, resembling this disorder pathologically, were found in the English and American literature, but none of the changes characteristic of beriberi was found in other organs, nor was a history of dietary inadequacy proven in these cases. The case studied by Davis and Wolf was a 5½-months-old Negro male, in whom a diagnosis of Wernicke's encephalopathy and beriberi was made pathologically. It had not been suspected during the three days that such an infant was in the hospital.

His mother had mild symptoms of numbness and tingling in her hands during the preceding 18 months, which were aggravated during her pregnancy. A male sibling, 18 months of age, was normal. At the age of one month the patient developed a mild eczema which was treated with various local medications and at 9½weeks of age he was placed on a formula derived from soy beans. He took between 15 and 20 ounces of this formula per day. After preparing it the mother boiled it for two or three minutes and stored it in a thermos bottle, where it remained hot for the rest of the day. For the two months preceding his hospitalization he took about two teaspoonsful, daily, of carrots, squash, or sweet potato. He never took cereal, nor was he ever offered fruits or meats. No vitamin supplements other than ascorbic acid were given. On this regimen his eczema cleared two weeks before he was hospitalized.

He then developed increasing irritability, restlessness, and occasional vomiting. During the days immediately preceding hospitalization he become somnolent but irritable during his waking intervals. There was no hoarsness, impairment of function of extraocular muscles, nor change in urinary output.

The essential physical findings were pallor and slight dehydration. The only neurologic finding was persistent ankle clonus. Laboratory examinations revealed essentially normal blood and urine. The spinal fluid was normal. The same dietary regimen was continued in the hospital. On the third day he became pale, semicomatose and had rapid breathing. Acetone was detected on his breath. Examination of the blood at that time revealed a serum chloride concentration of 116 mEq per liter and carbon dioxide combining power of 3.6 mEq per liter. The non-protein nitrogen was 111 mg. per cent and there was a 3+ reaction for acetone in the plasma. The blood pH was 7.1. He expired on the third hospital day.

The essential pathological findings were fatty degeneration of the myocardium, fatty infiltration of the liver and renal tubular epithelium, mild anasarca, visceral congestion, and subserosal hemorrhages. Microscopic examination of the central nervous system revealed acute degeneration of nerve cells and endothelial hyperplasia of capillaries in the gray matter in the walls of the third and fourth ventricles and in the periaquaductal region, including characteristic involvement of the corpora mammillaria. The role of thiamine deficiency in Wernicke's encephalopathy has been reviewed recently (*Nutrition Reviews* **11,** *204* (*1952*)).

Because these findings strongly suggested the diagnosis of thiamine deficiency, the dietary histories were reviewed in considerable detail. The mother's diet was considered adequate. Two complete neurologic examinations had been within normal limits and on one occasion the pyruvate concentration in the mother's blood was 1.08 mg. per 100 ml., which was considered as high normal. A

thiamine tolerance test done at that time was within normal limits.

Thiamine determinations by the thiochrome procedure were performed on a soy bean formula diluted and heated exactly as had been done by the patient's mother. The initial concentration of thiamine was 3.84 micrograms per 100 ml. and decreased to 2.76 micrograms per 100 ml. after 24 hours in the thermos bottle. These values are lower than the average thiamine content of whole cow's milk, which is 45 micrograms per 100 ml., or that of undiluted evaporated milk, which is 50 micrograms per 100 ml.

The authors concluded that the reduction in thiamine concentration in the commercial soy bean formula was to be expected because of the extent of heat processing for sterilization and hypoallergenic treatment. The approximate thiamine content of this infant's diet for the two months preceding death was estimated as between 16.5 and 32 micrograms per day. This was considerably below the minimal requirement of 140 to 200 micrograms per day, as determined by L. E. Holt, Jr. *et al.* (*J. Nutrition* **37,** *53* (*1949*)).

This very low thiamine intake in a growing child for a three month period, associated with pathological changes compatible with those seen in man and animals in which a diagnosis of thiamine deficiency had been established, certainly suggests that this infant was deficient in this vitamin. The absence of other significant findings would suggest that this deficiency proved fatal. The potential dangers of stringent dietary restrictions in the treatment of allergic conditions in small infants is emphasized in this case report. It should be pointed out that this child's eczema disappeared over one week before the onset of his fatal illness. In view of the wide-spread use in this country of multiple vitamin preparations, it seems almost ironic that their omission in this infant, who was ingesting an obviously inadequate diet, should have occurred.

This situation recalls to mind a case of avitaminosis A reported by D. Cornfeld and R. C. Cooke (*Nutrition Reviews* **10,** *327* (*1952*)) which also occurred in a 5½-months-old infant placed on a purified diet for the treatment of allergy to cow's milk. (See also M. H. Bass and J. Kaplan, *J. Pediat.* **47,** *690* (*1955*); I. J. Wolf, *J. Am. Med. Assn.* **166,** *1859* (*1958*)). The continued appearance of similar instances in the literature would suggest that, at the present time, the most clear-cut examples of nutritional deficiency in infants in this country occur in allergic children whose diets have been modified extensively during the course of their treatment.

POULTRY BREED DIFFERENCES IN SULFUR-AMINO ACID METABOLISM

In recent years there has been considerable interest in sulfur-amino acid supplementation of practical poultry rations. Experimental results have not, however, indicated any uniform or consistent growth response to such supplementation.

An interesting recent observation in Australia has emphasized breed differences in response to methionine supplementation (M. W. McDonald, *Aust. J. Agr. Res.* **8,** *587* (*1957*)). When a diet based on wheat meal, meat meal, and whey powder was supplemented with DL-methionine, white leghorn chicks and Australorp chicks differed considerably. The former exhibited growth response and the latter exhibited growth depression. The difference was found to be sex-linked; the ability of leghorns to use methionine was completely dominant.

Additional nutritional, as well as biochemical, studies have been reported more recently (McDonald, *Ibid.* **9,** *161* (*1958*)). The addition of 0.078 per cent of cystine to the basal diet increased the weight gain of Australorps by 5.3 per cent and of white leghorns by 4.3 per cent. A methionine sup-

plement (0.10 per cent) to the same basal diet increased the weight gain of leghorns by 5.4 per cent, but produced a reduction in weight gain of 5.4 per cent in the Australorp chicks. To supplement the results of the nutritional studies, the *in vivo* production of cysteine from methionine was investigated in leghorns and Australorps by determining the non-protein cysteine (both cysteine and reduced glutathione) content of the livers after intraperitoneal methionine administration. In leghorns the liver cysteine content (micrograms per gram) rose significantly from a normal level of 74.0 to 89.2 and 100.5 one hour after injection of 10 and 20 mg. of methionine, respectively. In the Australorp breed neither level of methionine produced a significant increase in liver cysteine content (normal level, 96.0 micrograms per gram). A second experiment revealed a 35.3 per cent increase in liver cysteine content in leghorns, and only an 8.3 per cent increase in Australorps, following the intraperitoneal injection of 20 mg. of methionine.

Methionine was also administered via the crop with essentially the same breed responses in liver cysteine content as was observed when the methionine was injected, although the response did not reach maximum until three hours after treatment.

The response of the Australorp breed to dietary cysteine, but not to methionine, suggests that the basal diet was deficient in a nutrient for which cystine was a precursor. Failure of this breed to respond to methionine, unlike the leghorn breed, suggests that Australorps were unable to use methionine for synthesis of the deficient nutrient. McDonald concludes that the deficient nutrient is cysteine and that this amino acid or its precursor, cystine, must be a dietary essential for the Australorp breed.

The failure of Australorps to produce significant increases in liver cysteine following methionine injection is evidence of a metabolic block. The fact that small, though non-significant, response to methionine was observed suggests that the synthetic mechanism may not be completely blocked.

McDonald notes the interesting observation that the gene, or group of genes, which control cysteine synthesis and the gene which determines early feathering (A. S. Serebrovsky, *Amer. Natur.* **56,** *571 (1922)*) are both located on the x-chromosome. However, mixed matings revealed that cross bred cockerels from the two breeds were found to be late feathering but cysteine normal. Hence the late feathering must be due to some other mechanism than ability to synthesize cysteine. These interesting results again focus attention on the impact of heredity on nutritional requirements.

GROWTH OF ANIMALS FED RAW SOYBEANS

It has been recognized, since the work of T. B. Osborne and L. B. Mendel, that raw soybeans in a ration produce poor growth in rats and that the growth-promoting activity of the soybeans was improved after autoclaving (*Nutrition Reviews* **11,** *253 (1953)*). In 1936 experimental work provided a suggestion for the mechanism whereby autoclaving improved the biological value of soybeans. The work of J. W. Hayward, H. Steenbock and G. Bohstedt (*J. Nutrition* **12,** *275 (1936)*) showed that the addition of 0.3 per cent of cystine to the raw soybean diet produced a weight gain of 41 g. in weanling rats over a 56-day period. This compared with a weight gain of 19 g. in the rats fed the raw soybeans. In spite of the marked increase in weight gain of the cystine-supplemented rats, their weight gain was still short of the 50 g. gain made by the rats fed autoclaved soybeans.

Considerable work has been done to elucidate the reason why cystine supplementation improved the biological value of raw soy-

beans. An explanation appeared at hand when it was shown that raw soybeans contained a trypsin inhibitor and that the activity of the latter was destroyed on heating (W. E. Ham and R. M. Sandstedt, *J. Biol. Chem.* **154,** *505 (1944)*). Additional work by R. Borchers, C. W. Anderson and F. E. Mussehl (*Arch. Biochem. Biophys.* **19,** *317 (1948)*) showed that a crude preparation of trypsin inhibitor prepared from raw soybeans, when added to a ration containing autoclaved soybeans, produced a weight gain in weanling rats of 60 g. over a period of 42 days. The control animals on the autoclaved ration gained 74 g. However, purification of the trypsin inhibitor decreased the above growth-inhibiting activity. On the basis of this work, Borchers and co-workers (*Loc. cit.*) suggested that the growth-depressing action of the crude trypsin inhibitor extracted from raw soybeans was due to a toxic substance.

Work on the latter suggestion led to the isolation of a toxic protein by I. E. Liener and M. J. Pallansch (*Nutrition Reviews* **11,** *253 (1953)*) who named it "soyin." Addition of this product to the autoclaved soybean ration reduced the weight gain in rats. The reduction, however, was not as great as that seen when rats were fed the raw soybeans.

The explanation for the poor growth of animals fed raw soybean rations is becoming more complex with additional research. This is shown by the recent reports of R. Borchers and D. Mohammad-Abadi (*J. Nutrition* **59,** *435 (1956)*). They attempted to evaluate the role that intestinal putrefaction plays in the differential growth produced by raw and autoclaved soybeans. To this end, they used the urinary excretion of indican as an indicator of intestinal putrefaction. When weanling rats were fed a raw soybean ration supplemented with methionine (this amino acid can replace cystine in increasing the growth of rats fed raw soybeans) and all the necessary minerals and vitamins, they gained weight at the rate of 2.9 g. per day and excreted 3.8 mg. of indican per 100 g. of rat per day. Autoclaving the soybeans increased the growth rate to 4.2 g. per day and reduced the indican excretion to 0.9 mg. The inverse relation between weight gain and urinary indican excretion did not hold when 5 per cent of a crude trypsin powder (to overcome the activity of the anti-trypsin factor in raw soybeans) was added to the raw soybean ration. In that case, the rats gained 4.0 g. and excreted 40 mg. of indican. The addition of the crude trypsin powder to the autoclaved soybean ration had no effect on either weight gain or indican excretion.

Even though the indican study strongly suggested that the putrefaction processes in the gastrointestinal tract did not account for the difference in the growth on the raw and heated soybean ration, R. Borchers, D. Mohammad-Abadi and J. M. Weaver (*J. Agr. Food Chem.* **5,** *371 (1957)*) extended their studies to the effect of a variety of antibiotics in overcoming the poor weight gains seen on the raw soybean diet. They used four female and four male weanling rats in each of their groups. The basal diet consisted of soybean oil meal (autoclaved or raw), DL-methionine, and the necessary salts, vitamins and carbohydrates. When either penicillin (at a level of 0.01 and 0.1 per cent) or streptomycin (at levels of 0.01 to 0.4 per cent) was added to the diet, the growth rate of the rats fed the raw soybeans was, in most cases, 70 to 80 per cent that of the group fed the autoclaved beans. This was the same as that secured on the unsupplemented raw soybean diet. There were a few experiments in which the individual antibiotics produced almost as good growth as that seen on the autoclaved soybean diet. In the latter cases, there was no correlation between the level of the antibiotic used and the improvement in growth rate. When penicillin and streptomycin were used together in the same ration (0.01 and 0.1 per cent, respectively), the growth rate of the rats on the raw soybean diet was from 89 to 106 per cent of the group fed the autoclaved soybeans. The growth rates were measured over a 20-day period.

Although there is no agreement as to the mechanism whereby dietary antibiotics produce a growth-stimulatory effect, it is fairly well recognized that the action involves a change, either in the flora of the gastrointestinal tract and/or in the intestinal wall. The latest reports from the group at the University of Nebraska (Borchers, Mohammad-Abadi and Weaver, *loc. cit.*) strongly suggest that the raw soybean diet has some adverse effect on one or both of the above.

An additional complication arises from the observations that even though chicks do not utilize raw soybeans as well as the heated beans, hens are able to use both forms equally well for egg production. H. J. Almquist and co-workers (*J. Nutrition* **24,** *385 (1942)*) found that when chicks were fed a diet containing raw soybeans, they gained weight at a rate arbitrarily designated as 2.8. Addition of 0.5 per cent of methionine to the ration increased the rate to 6.9, which was still less than the 7.9 for the group fed the ration containing heated soybeans. Earlier than this, it had been shown by A. E. Tomhave and C. W. Mumford (*Univ. Delaware Agric. Exper. Sta. Bull. No. 197 (1936)*) that when hens were fed raw soybeans in their ration, they produced as many eggs as those fed the heated meal.

The work with laying hens has been extended by H. Fisher, D. Johnson, Jr. and S. Ferdo (*J. Nutrition* **61,** *611 (1957)*). These workers fed groups of eight hens a ration containing glucose, corn oil, adequate minerals, vitamins (including vitamin B_{12}), methionine and 30 per cent of either raw or heated soybean meal (providing rations with 15 per cent protein). Over a three-week period, the hens fed the raw soybean ration produced as many eggs (21.8, 17.9 and 18.0 per 28-day period in three experiments) as the birds fed the autoclaved soybean rations, (23.2, 17.2, 18.9 eggs). In the absence of supplemental methionine, the hens fed the raw soybean ration laid 6.5 eggs in 21 days whereas those fed the heated soybean ration laid 8.8 eggs. Adding a crude trypsin inhibitor to the heated soybean ration, surprisingly enough, increased egg production to 11.8. The addition of methionine (at a level of either 0.1 or 0.2 per cent) produced no further increase in egg production, even in the absence of the inhibitor. These findings suggest that the laying hen is insensitive to the toxic and trypsin-inhibiting principles in raw soybeans.

Similar experiments with growing chicks showed that the raw soybean ration (supplemented with methionine and vitamin B_{12}) produced a growth inhibition of 10.0 to 15.2 per cent over a three-week period when compared with the heated soybean ration. There was an inverse relation between the level of protein (the soybean protein ranged from 20 to 26 per cent of the ration) and the growth inhibition. The above workers (Fisher, *et al., Ibid.*) stated that the differential growth produced by raw and heated soybeans was apparent in the chick through the fourteenth week of life.

The animal studies on the biological value of soybeans not only is of interest from a theoretical standpoint but also is of considerable practical importance. J. M. Hundley (*Ann. N. Y. Acad. Sci.* **69,** *1042 (1958)*) has pointed out that for large areas of the world, vegetable proteins will continue to be the primary form available. In many of these regions, soybeans may offer plant proteins of high biological value. The research work described above will aid in answering some of the questions that have been raised about their nutritional application.

TETRACYCLINE FLUORESCENCE OF BODY TISSUES AND TUMOR TISSUES

During the course of clinical studies conducted on cancer patients with a riboflavin antagonist, an incidental finding was the observation of a persistent yellow fluores-

cence under ultraviolet light in the tumor tissue of the patients who had received tetracycline during that period.

This observation prompted further investigation of this phenomenon, using mice bearing transplanted tumors (D. P. Rall, *et al., J. Nat. Cancer Inst.* **19,** *79 (1957)*). Following the administration of the riboflavin analogue, a transient fluorescence under ultraviolet light was exhibited by all the tissues. This disappeared completely within a few hours. In contrast, following the administration of single doses of either of three tetracycline compounds (tetracycline, chlortetracycline, oxytetracycline) there was a transient bright yellow fluorescence of all the tissues for a few hours, which persisted in the bones, teeth and tumor tissues where it remained essentially undiminished until the death of the animals (as long as 10 to 20 days later). When the tetracycline was administered prior to the fifth day of the implanted tumor growth, no fluorescence was seen in the tumor. When it was administered after the sixth day, on the other hand, it uniformly resulted in the production of fluorescence in the tumor implants. "Fluorescent material appeared and persisted in 14 of 19 different experimental neoplasms in mice" after tetracycline administration. No effect on the growth of these tumors was observed grossly following tetracycline administration, although that aspect was beyond the scope of the study described above.

Further investigation was carried out with special reference to the skeletal localization of the fluorescent material (R. A. Milch, *et al., J. Natl. Cancer Inst.* **19,** *1, 87 (1957)*). Sprague-Dawley rats, CF mice and New Zealand white rabbits were injected with tetracycline. The lower end of the femur was selected as the bone of observation at the arbitrary time of four hours after the administration of tetracycline. Fresh sections of undecalcified bone were examined under a standard wavelength ultraviolet light (3660 Å) and were photographed through one microscope with one camera under standardized conditions of exposure time and type of film used. It was observed that, once deposited in the bone, the fluorescent material did not appear to be displaced throughout the remainder of the ten-week experimental period. The intensity and distribution of the fluorescence was different in young and adult bones, which could be readily differentiated. Also, there seemed to be an increased concentration of this fluorescence along the periosteal and endosteal surfaces, suggesting a direct relationship of this localization to the blood supply.

It is concluded from the selective distribution and sharp demarcation of the areas of localization of the fluorescent material in bone that physicochemical phenomena, other than the availability and simple diffusion of the tetracycline compound, are involved. It is supposed that tetracycline or a metabolic derivative is capable of being bound for relatively long periods of time into regions of active bone proliferation through some type of surface reaction. This is also probably a characteristic of the naphthacenecarboxamide nucleus of tetracycline and not affected by substitution of a chlorine atom in position 7, or a hydroxyl radical in position 5, since tetracycline, chlortetracycline and oxytetracycline produce essentially the same observed effects.

ERUCIC ACID AND FERTILITY

Normal reproduction in the female is known to be dependent on adequate nutrition. A large number of nutritional deficiencies have been shown to interfere with implantation, gestation, and lactation in experimental animals. In contrast, spermatogenesis in the male is relatively resistant to nutritional influence. Vitamin E deficiency

results in irreversible testicular degeneration in the rat, while vitamin A deficiency results in testicular damage which is reversible under certain conditions (K. E. Mason, *Am. J. Anat.* **52,** *153 (1933)*).

The importance of essential fatty acids in testicular development of rats was pointed out by H. M. Evans, S. Lepkovsky and E. A. Murphy (*J. Biol. Chem.* **106,** *445 (1934)*). Male rats, fed a fat-free diet, became sterile and histological study of the testes demonstrated the absence of mature sperm cells. Supplementation of the diet with small quantities of essential fatty acids prevented these changes. Fertility could be restored in the fat deficient rats by re-feeding the essential fatty acids, even after considerable testicular damage had occurred.

The relationship of essential fatty acids to testicular function is again indicated by the report of K. K. Carroll and R. L. Noble (*Canad. J. Biochem. Physiol.* **35,** *1093 (1957)*). A study was made of the effect of erucic acid on fertility of male and female rats. Erucic acid is a fatty acid with 22 carbons and one double bond. It is found in high concentration in rapeseed oil. It has been suggested that this acid may serve as a metabolic antagonist of the essential fatty acids.

Erucic acid and various other fats were added to a stock diet for growing male rats. After varying periods on the diet, the animals were mated with female rats. After receiving a diet containing 15 per cent erucic acid for approximately three months, male rats were sterile. The feeding of 10 per cent erucic acid for four to five months also produced sterility in male rats. In contrast, fertility was normal in male rats receiving 25 per cent rapeseed oil, or 15 per cent oleic acid. The rats receiving 25 per cent rapeseed oil were ingesting a quantity of erucic acid which, if fed alone, would have resulted in sterility. The authors postulate that the other fatty acids present in the rapeseed oil may have counteracted the deleterious effect of the erucic acid which it contained.

The feeding of erucic acid led to a reduction in the size of the testes, but was without significant effect on the weight of the seminal vesicles or prostate. Histological study of the testes of erucic acid-fed rats revealed an accumulation of fluid around the tubules and the absence of mature sperm cells. The interstitial cells appeared to be little affected.

Erucic acid appeared to be much less effective in producing testicular change in mature rats, although some degeneration was observed after five months of feeding.

Observations were made on the recovery from testicular damage after cessation of the erucic acid feeding. When the erucic acid had been fed for more than five months there was no testicular recovery as judged by testicular size and by mating performance even after 16 weeks of feeding a normal diet. It appears that the testicular damage from erucic acid is essentially irreversible.

The feeding of 15 per cent erucic acid to growing female rats resulted in a high incidence of sterility. When implantation occurred, resorptions were frequent. The young born to erucic acid-fed rats were usually small, and many died within three weeks. These effects were essentially irreversible as judged by reproductive performance of female rats originally fed erucic acid and then transferred to a normal diet.

The results of these experiments demonstrate clearly that erucic acid exerts a deleterious effect on reproduction in both male and female rats. The results do not prove that these effects are due to a deficiency of essential fatty acids. In fact the erucic-fed rats exhibited none of the usual signs of essential fatty acid deficiency except testicular damage. Growth was normal and no skin changes were observed. Scaly dermatitis is a classical sign of essential fatty acid deficiency in rats.

If erucic acid exerts its effect on reproduction by acting as a metabolic antagonist to essential fatty acids, it should be possible to prevent the effect by the simultaneous feeding of essential fatty acids. Such experiments would be a logical extension of the present work.

CORTISONE EFFECTS ON BONE MINERAL METABOLISM

Cortisone is known to affect bone mineral metabolism in several ways. Clinically, prolonged treatment with cortisone, or one of the newer synthetic analogues, leads to the rarefaction of bone, particularly in the vertebral column and pelvis, in a substantial number of patients. Pathogically the bone spicules are reduced in number and thickness, but there is no evidence of increased osteoclastic activity. The changes in bone which occur in hyperadrenalcorticism have been attributed by F. Albright and E. C. Reifenstein (*The Parathyroid Glands and Metabolic Bone Disease*, Baltimore, Williams and Wilkins, 1948) to decreased formation of osteoid, the proteinaceous matrix on which bone salts are deposited. Androgenic and estrogenic steroids antagonize the osteoporotic effects of corticosteroids to a considerable extent. The clinical usefulness of these agents in patients receiving corticosteroid treatment has been denied by some authorities but reiterated by E. C. Reifenstein (*Metabolism* **7,** *78* (*1958*)).

Under certain circumstances corticosteroids inhibit the absorption of calcium from the gastrointestinal tract. Such an action may be evident in conditions where absorption of calcium is excessive, as in sarcoidosis or hypervitaminosis D. In sarcoid J. Anderson, C. E. Dent, C. Harper and G. R. Philpot (*Lancet* **267,** *720* (*1954*)) and P. H. Henneman and co-workers (*J. Clin. Invest.* **35,** *1229* (*1956*)) have shown that cortisone leads to a decrease in an elevated serum calcium by increasing the fecal excretion of calcium. The possibility has been suggested that hypercalcemia in sarcoidosis may be due to an endogenous hypervitaminosis D. The therapeutic action of cortisone could be attributed to antagonizing the intestinal actions of the vitamin according to this scheme. Further support for this concept is provided by the observation of R. C. Moehlig and A. L. Steinbach (*J. Am. Med. Assn.* **154,** *42* (*1954*)) that the tachysterol requirement of a hypoparathyroid patient was increased by cortisone. Also, H. Colcher, S. R. Drachman and D. Adlersberg (*Ann. Int. Med.* **38,** *554* (*1953*)) have found that cortisone may precipitate tetany in patients with sprue.

The antagonistic effects of cortisone and vitamin D on bone and serum citrate levels have been studied in rats by H. C. Harrison, H. E. Harrison and E. A. Park (*Proc. Soc. Exp. Biol. Med.* **96,** *768* (*1958*)). Their interest in this problem was stimulated by the accumulating evidence indicating an important role of citrate in bone mineral metabolism. In a review last year some aspects of this problem were discussed (*Nutrition Reviews* **15,** *188* (*1957*)). Both vitamin D and parathyroid hormone increase the concentration of serum and bone citrate under certain experimental situations. It has been suggested that the formation of citric acid locally in bone may be important in maintaining the concentration of serum calcium by solubilizing bone calcium and transporting it to the blood stream.

The observations of Harrison and coworkers have considerable bearing on this problem. First of all, they demonstrated that the levels of serum citrate, which are low in vitamin D deficiency in rats are further reduced by the administration of cortisol. In rats which were fed a low phosphorus diet, the levels of serum phosphorus of the rachitic rats treated with cortisol were lower than those of the untreated rachitic rats. Calcium levels in the serum were not significantly different. The histologic appearance of the tibias of the cortisol treated groups was remarkable in that the cartilage mass was found to be heavily calcified.

Further observations were carried out on vitamin D treated rachitic rats with and without cortisol treatment. The rats receiving Vitamin D alone exhibited the expected rise in serum and bone citrate. This rise was prevented when vitamin D was administered with cortisol. Serum calcium levels were equal-

ly high in the animals receiving cortisol plus vitamin D, but the serum phosphorus levels were lower. Despite these chemical findings, the calcification of cartilage was unimpaired, although the histologic pattern was somewhat distorted. The lowering in the serum phosphate level was attributed to increased deposition in cartilage and bone rather than to decreased absorption from the gut. The authors speculate that cortisol is associated with some change in cartilage matrix which results in local precipitation of bone salts.

Many of the experimental results can be explained by an inhibitory effect of cortisol on citrate formation in skeletal tissue. The lowered skeletal citrate would increase the concentration of unbound calcium ion and permit its precipitation in cartilage and bone, despite the extremely low level of serum phosphorus. With the high doses of cortisol employed, the inhibition of citrate production was not overcome by vitamin D. Increased gastrointestinal absorption of calcium probably occurred as evidenced by the increased serum calcium levels. With the data at hand it is not possible to say that the absorption of calcium in the presence of cortisol and vitamin D was entirely normal.

Another effect of cortisone, which is pertinent to the topic under review, concerns the effect of the hormone on phosphate excretion by the kidney. Both in human beings and dogs cortisone has been found to increase the urinary excretion of phosphate by depressing the reabsorption of phosphate by the renal tubules. A. Laron, J. D. Crawford and R. Klein, (*Proc. Soc. Exp. Biol. Med.* **96,** *649 (1958)*) have considered the possibility that the decreased phosphate reabsorption could be attributed to increased parathyroid activity. First they showed that cortisone did increase the clearance of phosphate in the normal rat, and that parathyroidectomy did not change the response in that there was an increase in phosphate excretion without alteration in serum phosphate level. Phosphate loading of the parathyroidectomized rats was carried out, and again cortisone increased the phosphate clearance. Starvation, however, was effective in blocking the renal action of cortisone.

It is clear from these experiments that the phosphaturic effect of cortisone is not mediated by the parathyroids, but the source of the excess phosphate appearing in the urine has not been accounted for. The authors suggest that, in view of the unaltered serum inorganic phosphate level, increased gastrointestinal absorption of phosphate had occurred. The failure to obtain a cortisone effect in starvation was ascribed to the endogenous hyperfunction of the adrenals recognized in this condition.

In summary, we can say that cortisone affects bone mineral metabolism at three sites, which are the gastrointestinal tract, bone itself and the renal tubule. The predominant effect is influenced by the experimental conditions and the species of animal under study.

TOXIC EFFECTS OF ANTIMETABOLITES

The extensive search for useful agents in cancer chemotherapy involves, on the one hand, the empirical screening in animal systems of vast numbers of synthetic chemical compounds and biological substances and, on the other hand, the purposeful synthesis of antimetabolites which resemble their natural analogues sufficiently to compete with them in biological systems. The ideal agent should act against tumor without significantly affecting the host.

Metabolic studies of such specific action by 6-mercaptopurine were reported by R. P. Heaney and L. P. Eliel (*Cancer* **9,** *257 (1956)*). Two children, aged three and one-half and four years, with lymphosarcoma

and acute lymphoblastic leukemia, respectively, had virtually complete hematological and clinical remissions on 6-mercaptopurine. Analysis of metabolic studies conducted during therapy revealed the persistence of normal anabolic activity in the non-tumor tissues of both children. Nitrogen, phosphorus and potassium were excreted in ratios approximated by the content of these chemicals in destroyed leukemic tissues. Calculations made from the observed balances demonstrated the persistence of positive nitrogen and phosphorus balance throughout the period of study. Cytochemical changes in biopsied tumor tissue showed a decrease in whole cell phosphorus and in the nuclear fraction nitrogen, phosphorus and potassium, attributed to a decrease of the content of tumor nucleic acid. The authors did not claim that 6-mercaptopurine had absolutely no effect on non-leukemic tissue citing, for example, a sustained drop of alkaline phosphatase from 5.7 and 5.5 Bodansky units per 100 ml. to 3.9 and 3.0 units. This was interpreted as an index of reduced osteoblastic activity. The calcium balances in these two cases were too variable to be analyzed, but data from another case revealed a concurrent rise in urinary calcium excretion during 6-mercaptopurine administration.

Since tumors originate from host tissues, antimetabolite effect upon tumor cannot be entirely specific, and all effective antimetabolites must necessarily be toxic to the host if the dosage is sufficiently high; 6-mercaptopurine is no exception to the rule. F. S. Philips, S. S. Sternberg, L. D. Hamilton and D. A. Clarke (*Cancer* **9,** *1092 (1956)*) compared the toxic effects of thioguanine (the 2-amino derivative of 6-mercaptopurine) to those of 6-mercaptopurine in mice, rats, cats and dogs. The agents both reversibly injure bone marrow. For thioguanine, the bone marrow aplasia, with hyperpyrexic toxemia and hemorrhage, is the main factor in resultant mortality, resembling the late bone marrow syndrome of median lethal total body irradiation. Fatal doses of 6-mercaptopurine, on the other hand, induce a more fulminating course due to lesions in the intestinal epithelium and liver. Dogs develop hemorrhagic diarrhea and jaundice, while rats show species-specific pulmonary, mediastinal and myocardial changes. Diarrhea with thioguanine usually occurs late. An interesting difference between the two drugs lies in the fact that repetitive doses markedly enhance the toxicity of thioguanine but not that of 6-mercaptopurine. The reasons for the various discrepancies between the effects of the two drugs are as yet obscure.

M. L. Murphy and D. A. Karnofsky (*Cancer* **9,** *955 (1956)*) studied the effects of antimetabolites upon the living embryo at various stages of development within the pregnant rat. Although innocuous to the mother, 2.5 mg. per kilo of azaserine markedly disturbed fetal development during the eighth to twelfth days of gestation but had no effect on the seventh, fourteenth, sixteenth or eighteenth days. Newborn embryos (21 days old) were resistant to 50 mg. per kilo of azaserine in that only two of eleven died. The chief deformities noted were harelips, cleft palates and other skeletal defects. The treated embryo weighed about 20 per cent less than the untreated. Aminopterin in doses of 0.2 mg. per kilo injected into pregnant rats was most toxic on the ninth to tenth days. The effect of aminopterin seemed largely an "all or none" action, in that either the fetus died early or survived to 21 days without major anomalies. Nitrogen mustard, which is not an antimetabolite, differed somewhat in its action upon the embryo. It was most toxic from the eleventh to the sixteenth days of gestation, producing frequent encephaloceles and defects in the cranial bones and ribs. The effects of nitrogen mustard are similar to radiation effects cited in the literature, with respect to the time of gestation and the ratio of teratogenic dose to maternal LD 50. In general, it seems that the embryo is resistant to noxious agents up to the time of implantation, namely

seven to eight days. During the critical phase of gestation, nine to thirteen days, the antimetabolites affect fetal growth and survival much more than nitrogen mustard or X-ray. However, the latter two agents affect fetal growth after the fourteenth day, when the fetus is resistant to antimetabolites. The authors relate this difference in effect to the fact that antimetabolite inhibition of nucleic acid synthesis may have its greatest effect at the time of rapid differentiation, while radiation has a less time-specific damaging action upon nucleoproteins. Of course, the obvious similarity of embryo in mother to tumor in host makes this system of interest as a screening device for chemotherapeutic agents.

The human hepatotoxic effect of N-methyl-formamide, a compound which seems structurally related to urea and urethane, has been studied by W. P. L. Myers, Karnofsky, and J. H. Burchenal (*Cancer* **9,** *949 (1956)*). This compound was found to inhibit the growth of transplantable sarcoma 180 in the mouse. Its action on tumor or toxicity could not be reversed by formate, acetate, glycine, pteroylglutamic acid or citrovorum factor. Although a trial of this drug in five patients with advanced cancer demonstrated no tumor-inhibiting properties, all five patients developed anorexia, nausea and vomiting, and laboratory evidence of liver toxicity with total oral doses of 0.1 to 4.0 g. per day or intravenous doses up to 2.0 g. daily for a total of two to 36 days. Elevation of bromsulfalein retention, elevated prothrombin time, elevated thymol turbidity and reduction in cholesterol esters and serum albumin were observed in various combinations. No mention is made of variations in bilirubin. One patient was obviously jaundiced but thought to have liver metastases. At autopsy, one patient showed microscopic evidence of hepatic lobular disorganization. In general, the alterations in liver chemistries were reversible after the drug was stopped. The demonstration of hepatotoxicity in humans bore out similar experiences in mice, dogs, and rats, although toxicity occurred at an unexpectedly low dose level in the human.

The toxicity of 6-diazo-5-oxo-2-norleucine (DON), an agent with some antitumor activity, is discussed by G. B. Magill, *et al.*, (*Cancer* **10,** *1138 (1957)*). Levels of 0/2 to 0/3 mg./kilo/day produced human toxicity, but divided doses were more toxic than single daily doses and doses at intervals of two to four days permitted tolerance of up to five times the quantity otherwise tolerated. The structure of DON is similar to that of azaserine (O-diazo-acetyl-α-serine), but DON is ten to 100 times as potent on a weight basis, perhaps partially because DON is not destroyed by liver enzymes. It causes azaserine-like gastrointestinal changes in animals, but is not as toxic to pancreas, liver and kidneys in mice, as is azaserine.

In humans, 52 of 63 patients had oral mucosal toxicity, manifested by redness, dryness, soreness and bitter taste, eventuating in ulceration. Thirty patients had mild diarrhea; 19 patients had easily controlled nausea and vomiting; ten patients had gastrointestinal bleeding (directly attributable to DON in two, one with duodenal ulcer, the other with hemorrhagic gastritis); five patients had abdominal pain and distention due to ileus. Hematologically, 15 patients had a leukopenia of less than 4,000, usually due to relative neutropenia; seven patients had platelet counts less than 100,000. In ten of the 15 patients, the hematological changes were transient. In the others the longest duration of changes before deaths due to other causes was three weeks. Elevated blood urea nitrogen was seen in ten patients; the mechanism was obscure. Mental changes were seen in two patients with Hodgkin's disease.

The mucosal toxicity of DON could be reversed by 400 to 500 mg. per day of adenine or by 800 mg. per day of 4-amino-5-imidazole carboxamide, a purine precursor. Hematologic toxicity was not reversed by these substances, however.

IRON METABOLISM IN PROTEIN DEFICIENCY

Prolonged protein deficiency in experimental animals results in moderate anemia which is microcytic and hypochromic (*Nutrition Reviews* **12,** *341 (1954)*). This is the type of anemia which is seen in iron deficiency. In view of the fact that the transport, storage and biologic utilization of iron requires its conjugation with a protein, it might be expected that protein deficiency would lead to abnormalities in iron metabolism.

The reports of W. F. Bethard, *et al.* (*Blood* **13,** *146 (1958);* **13,** *216 (1958)*) indicate that quite early in protein deprivation the rat exhibits deranged iron metabolism. All experiments were conducted with young adult Sprague-Dawley rats. They were given a nutritionally adequate purified diet containing 22 per cent casein, or this same diet with casein replaced with dextrin. The latter diet was essentially protein-free. Another group of rats, given a diet of laboratory chow, behaved quite similarly to the animals given the complete purified diet.

After 35 days on their respective diets the animals were injected with the radioactive isotope of iron, Fe^{59}. The animals were then sacrificed at various time intervals between 3 and 72 hours, and the amount of Fe^{59} in the various tissues was determined.

The Fe^{59} disappeared rapidly from the plasma of both normal and protein-deficient rats, with little difference between the two groups. There was a striking effect of protein deficiency on the incorporation of the iron into bone marrow. Three hours after injection marrow from normal rats contained 60 per cent of the injected dose, while marrow from protein-deficient rats contained only 5 per cent of the injected dose. Thus it is apparent that the rate of entry of iron into bone marrow is severely depressed in protein-deficient rats. As would be expected in view of the bone marrow results, protein deficiency severely depressed the incorporation of iron into peripheral erythrocytes. Similar results were obtained with spleen; much less iron was incorporated by the protein-deficient rats. Thus, relatively early in protein deprivation the incorporation of iron into hematopoietic tissues is severely depressed.

Opposite results were obtained in the liver. Three hours after injection the livers from protein depleted rats contained 46 per cent of the dose, while livers from normal rats contained only 9 per cent of the dose. This may indicate that the inability of the hematopoietic tissue of protein deficient rats to utilize iron results in its accumulation in the liver.

The second series of experiments was designed to study the influence of protein deprivation at earlier time intervals on the incorporation of iron into peripheral erythrocytes. The rats were given the same protein-deficient and normal purified diets used in the earlier experiments. At five-day intervals the animals were injected with Fe^{59}. Five days after the injection the animals were sacrificed, and the Fe^{59} content of erythrocytes was determined.

Hemoglobin concentration, erythrocyte concentration, and hematocrit were slightly reduced after 35 days of protein depletion. However, blood volume of protein-deficient rats was reduced below normal, so that after 35 days of feeding the protein-deficient rats contained less than one half the normal number of total erythrocytes. It then appears that anemia may develop quite early in protein depletion, when the total blood volume is taken into consideration.

The uptake of Fe^{59} by erythrocytes was depressed essentially to zero after ten days of feeding the protein-deficient diets. When the deficient rats were given a complete diet the uptake of Fe^{59} by erythrocytes was restored to normal after ten days of feeding.

The results of these experiments indicate that iron metabolism is severely deranged quite early in protein deprivation. There is a reduction in the uptake of iron by all hematopoietic tissues. This may reflect a

requirement for protein for normal iron transport, and the effects of protein deficiency on iron-containing enzymes should be of considerable interest.

VITAMIN B_{12} AND PROTEIN SYNTHESIS

For several years biochemists have been searching for an explanation of the profound effect that vitamin B_{12} exerts on the growth of animals. Such studies have indicated a possible role of the vitamin in nucleic acid metabolism, in the metabolism of methyl groups, in the chemistry of enzymes with sulfhydryl groups, and in the formation of numerous enzymes in the liver (*see Nutrition Reviews* **14,** *282* (*1956*) for detailed references).

Workers at the University of Illinois have reported evidence suggesting that vitamin B_{12} may play a key role in protein synthesis (S. R. Wagle and B. C. Johnson, *Arch. Biochem. Biophys.* **70,** *619* (*1957*)). In preliminary studies, they found that when glucose, or serine labeled with C^{14}, was injected into vitamin B_{12} deficient rats or pigs, their liver proteins contained less of the radioactivity than did the liver proteins from normal animals.

In an extension of this work to *in vitro* studies, S. R. Wagle, R. Mehta and B. C. Johnson (*J. Biol. Chem.* **230,** *137* (*1958*)) have studied the synthesis of proteins by tissues from normal and vitamin B_{12} deficient rats. Weanling male rats were fed a diet composed of 72 g. full-fat soya flour, 22 g. lactose, 3 g. salts, 0.5 g. choline chloride, 0.1 g. DL-methionine, and all vitamins except vitamin B_{12}. Control rats were given the vitamin in the diet. After ten weeks on the diet, the deficient rats averaged 108 g. in weight and the control animals averaged 203 g. The latter is about one-half optimum growth for rats on a good diet. The deficient animals had lower red blood cell counts and lower hemoglobin concentrations.

At this time two animals from each group were killed; their livers and spleens were removed, homogenized separately, and centrifuged at high speed to separate the mitochondria (in the precipitate) from the microsomes (in the supernatant). The incorporation of amino acids into protein, presumably representing protein synthesis, was then determined by the procedure of Keller and Zamecnik (*J. Biol. Chem.* **221,** *45* (*1956*); *Nutrition Reviews* **15,** *55* (*1957*)). In the incubation experiments the supernatant fraction, containing microsomes, was mixed with several co-factors and either methionine, alanine or phenylalanine, each labeled with C^{14}. After one hour at 37° the total protein was precipitated with trichloracetic acid and washed several times to remove glycogen, nucleic acids and free amino acids. The radioactivity in the protein was then determined.

When a single amino acid, methionine labeled in the methyl group, was incubated with either liver or spleen microsome preparations from vitamin B_{12} deficient rats, the incorporation of radioactivity remained essentially constant as the amount of methionine was increased. With microsomes from normal rats, however, the amount of methionine incorporated into protein was three to five times that of the microsomes from the deficient rats. With increasing amounts of added methionine there was a progressive increase in incorporation, until a constant amount was reached at the higher levels. This experiment suggests that the ability to incorporate amino acids into protein is lost in vitamin B_{12} deficiency.

In the next experiment, the amount of methionine in the incubation mixtures was kept constant, and the concentration of added vitamin B_{12} was varied from zero to 50 millimicrograms per flask. Under these conditions, the microsome preparations from normal rats showed very little increase in incorporation of methionine with additional vitamin B_{12}. The preparations from deficient rats, with no added vitamin B_{12}, had about one-third the activity of the cor-

responding normal preparations. As vitamin B_{12} was added to these samples, however, there was a progressive increase in incorporation of radioactivity. The highest level of added vitamin gave an activity about two and one-half times that of the flask with no vitamin B_{12}. This highest activity, however, was less than that produced by normal rat preparations without added vitamin B_{12}.

A second experiment identical to the above, except that 2-C^{14}-alanine was used in place of methionine, produced similar results. This would indicate that the phenomenon being studied was not peculiar to one amino acid.

In a third experiment, a mixture of 20 amino acids, one of which was 3-C^{14}-phenylalanine, was incubated with microsome preparations from normal or vitamin B_{12} deficient rats. The incorporation of radioactivity was about four times that found in previous experiments when only a single amino acid (methionine or alanine) was used. The authors interpret this as indicating that a pool of amino acids is required for protein synthesis. Their experiment does not appear to permit this hypothesis, however, since they did not determine the amount of incorporation when phenylalanine was the single substrate. E. B. Keller and P. Zamecnik (*J. Biol. Chem.* **221,** *45* (*1946*)) were not able to stimulate the incorporation of a labeled amino acid by the addition of 17 other common amino acids.

By the use of Co^{60}-labeled vitamin B_{12} administered to a rat, Wagle and associates determined that about 40 per cent of the vitamin B_{12} in the liver was in the microsomes. About half as much was in the supernatant, and lesser amounts were in the mitochondria and nuclei. In earlier work on protein synthesis by cell fractions, M. D. Hoagland, E. B. Keller and P. Zamecnik (*J. Biol. Chem.* **208,** *365* (*1956*)) had shown that the synthetic activity was due to an enzyme in the supernatant acting together with the microsomes.

In order to see if this active enzyme fraction had an affinity for vitamin B_{12}, the Illinois group (*Arch. Biochem. Biophys.* **72,** *241* (*1957*)) have carried out further studies in which radioactive cobalt was incubated with microsomes or the supernatant fraction prepared from vitamin B_{12} deficient rats. Various co-factors known to be essential in protein biosynthesis were also included. Analysis of the active enzyme fraction (called B_{12}-enzyme) showed that the labeled vitamin B_{12} had been taken up in a ratio of 1 to 2 for the supernatant and microsome enzymes, respectively. This was the same ratio as observed (above) in the intact animal. The radioactivity could not be washed out with various treatments, nor could it be removed by dialysis.

These studies appear to be a very significant advance in our knowledge of the metabolic role of vitamin B_{12}. If, as the authors postulate, vitamin B_{12} is necessary for the synthesis of body proteins, then the vital nature of this vitamin in growth will be apparent. Before accepting such a critical function for vitamin B_{12}, however, verification and extension of these studies will be necessary. It will be important, for example, to show that other B vitamin deficiencies do not produce similar biochemical changes in liver and spleen. Wagle, Mehta and Johnson did show that vitamin A deficient rat livers do not have impaired protein synthetic activity. More careful control of the nutritional aspects (for example, the use of paired feeding) is necessary. Conclusive evidence that the *in vitro* biochemical procedure is actually measuring protein synthesis would appear to be essential. A stimulation of research activity in this field will undoubtedly follow these challenging reports.

EFFECTS OF GROWTH HORMONE FROM HUMAN PITUITARIES

An active growth promoting hormone has been known to be produced by the anterior portion of the pituitary gland. Purified growth hormone isolated from beef pitui-

taries has been found to be effective when injected in rats. However, when such a preparation has been injected into man its effects have usually been either negative or inconclusive.

C. H. Li and H. Papkoff (*Science* **124,** *1293* (*1956*)) have noted that the growth hormone obtained from beef and man differ in chemical properties. The differences include such physiochemical measurements as sedimentation and diffusion constants, isoelectric point, molecular weight, and differences in amino acid pattern. It was postulated that, because of the chemical differences in the beef and human growth hormone, biological differences might also be expected. These physiochemical differences might explain why beef growth hormone was not effective in man.

Recently, D. Ikkos, R. Luft and C. A. Gemzell (*Lancet* **1,** *720* (*1958*)) reported preliminary studies in which purified growth hormone isolated from human pituitaries was administered intravenously to human subjects. The hormone used was isolated from lyophylized human pituitaries according to the method proposed by Li and Papkoff. The preparation was found to be active on assay, using increase in width of the tibia of hypophysectomized rats as an index. Thus, the human growth hormone is effective in promoting growth in rats but the hormone isolated from beef pituitaries is not effective in man. Using electrophoresis, it was found that the purified growth hormone moved as a single component and no material was left at the site of origin.

To test its biologic activity, the hormone was given to two male volunteers. The first individual was 35 years old and had rheumatoid arthritis which was clinically inactive for the last eight years. The other individual was 45 years old and had been treated previously for obesity. He weighed 202 pounds and was 5 feet, 11 inches tall. Other than the size of the second subject, no information as to the nutritional status of these individuals was given. Each subject received 10 mg. of the hormone per day in the form of a single intramuscular injection. The first subject received the growth hormone for 12 days and the second received it for 16 days.

During the periods in which they were getting the growth hormone, both subjects had positive nitrogen balances. The first individual retained approximately 3.4 g. of nitrogen per day and the second retained 2.5 g. per day for the longer period. The authors calculated that each of the men had formed about 1.3 kg. of fat-free protoplasm during the hormone injections. In this preliminary note the nitrogen balances were given for each individual, but the nitrogen intake was not. The magnitude and fluctuation of this value would be of interest. In addition to positive nitrogen balance it was found that potassium and phosphorus were also being retained. The amount of potassium stored appeared to be in excess of nitrogen if protein synthesis were to account for all the electrolyte. However, the amounts of phosphorus and nitrogen stored were in agreement with the calculated amounts for tissue fabrication. The excess potassium, these investigators believe, could be accounted for by the formation of 1.3 and 0.5 kg. of wet glycogen by the two subjects.

While they were receiving the growth hormone, both individuals showed negative calcium balances when only urine was considered, but due to the changes in fecal calcium one subject had a daily positive balance of 54 mg. and the other a daily deficit of 121 mg. for the 16-day period. Small daily positive sodium (20 to 25 mEq) and smaller positive chloride balances were noted in both individuals when they were given the growth hormone.

Nitrogen equilibrium was attained during the four-day period in these subjects when the injections of the growth hormone stopped. The potassium balances during the period when the injections ceased changed to about negative 10 mEq per day. Negative phosphorus balances of about 100 mg. per day were also found when the growth hormone injections were stopped.

The subject with clinically inactive rheu-

matoid arthritis had a persistent creatinuria which disappeared during the 12 days when the growth hormone was given. After the administration of the hormone was stopped the creatinuria increased in amount, and in four days attained levels over twice the control values reported. The increased creatinuria persisted at about this level for the next 12 days, at which time the study ended. The injection of growth hormone in this individual also caused an increase in the amount of acetone in the urine from a control value of about 20 mg. to a maximum of almost 160 mg. per day. The acetonuria disappeared promptly upon stopping the hormone injections. The other subject did not show acetonuria during the time he received the growth hormone.

In a second preliminary communication R. Luft, D. Ikkos, C. A. Gemzell and H. Olivecrona (*Lancet* **1,** *721 (1958)*) have reported studies concerning the diabetogenic effect of administered growth hormone isolated from human pituitaries. The diabetogenic effect of administered growth hormone isolated from animal pituitaries has been established in animals. These investigators have studied the effects of giving 10 mg. per day of purified human growth hormone to three diabetic patients who had been hypophysectomized earlier in the course of treating their vascular complications (retinopathy, nephropathy, and neuropathy). One of the patients had been operated upon four and a half years previously and the other two had been operated upon two and a half years earlier. All patients were being maintained on insulin. After injecting the growth hormone two of the patients had immediate deterioration of their diabetic state with increased blood sugar levels, glycosuria, and ketonuria. Extra insulin was given but the rapid deterioration of the clinical picture 36 hours after the injection of the growth hormone caused them to stop all further injections. Another patient showed a similar reaction, though at a much slower rate. After receiving the growth hormone for 12 days increases in the blood and urine sugar levels and urine acetone concentration were noted, and thus it was felt necessary to stop the study.

This group of investigators has noted that the injection of growth hormone isolated from human pituitaries will cause a positive nitrogen balance along with the retention of potassium and phosphorus in two male subjects. Also, when given to a diabetic individual maintained on insulin it has a pronounced diabetogenic effect, increasing the levels of sugar in both the blood and urine and also the acetonuria. It is not definitely known at present whether these two actions are the properties of the purified growth hormone or whether they are due to different ententies. The attempted correlation of the differences in physiochemical properties of these hormones with differences in biologic activity is intriguing and offers great research possibilities.

NOTES

HOT WEATHER FEEDING OF CHILDREN AND INFANTS

Scientific information on the effects of climate on the dietary requirements of infants and children is apparently quite limited. Together with accumulated experience by pediatricians practicing in areas where prolonged periods of hot weather occur, this information has been reviewed by F. A. Norman and E. L. Pratt (*J. Am. Med. Assn.* **166,** *2168 (1958)*). The available studies suggest that during hot weather the nutrient requirements of infants and children are not increased, with the exception of sodium, chloride, and water.

Sweat losses averaging 56 cc. per kilogram per 24 hours have been measured in normal infants at an environmental temperature of

91° and low relative humidity. Sweat losses as high as 100 cc. per kilogram of body weight per day have been found in infants ill with diarrheal disease under similar conditions (*Nutrition Reviews* **10,** *293 (1952)*). Norman and Pratt point out that the greatest efficiency in restoring these losses is achieved by both infants and adults when the water losses are replaced hour by hour, although acclimatized individuals voluntarily accumlate slight water deficits during the day and make up these deficits at meal times and during the evening.

The amount of sodium and chloride lost is proportionately less than the water loss because of the low concentration of both these elements in normal sweat. Norman and Pratt have estimated that the intake of sodium and chloride in ordinary diets is adequate for preventing sodium or chloride deficits in infants. With the use of table salt in ordinary amounts there appeared to be no need for specific administration of sodium chloride in older children, except under conditions of great physical activity associated with extremely copious sweating.

The presence of infection, recurrent vomiting, or diarrhea was frequently associated with sodium and chloride depletion and the administration of salt was clearly of benefit in such patients. Certain other conditions, especially pancreatic fibrosis, are associated with excessive sodium and chloride loss in sweat and serious salt depletion may occur very rapidly during hot weather. No evidence was found that the addition of sodium chloride to the diet of the normal infant was beneficial, and the authors come to the conclusion that an infant or child suffering from heat stress is probably ill and deserves further investigation.

Practical observations suggest that the natural reduction in caloric intake, and hence heat production (reflected in a slower gain of weight during hot weather), as well as the ingestion of cold foods and liquids may become perverted if these cold foods are high in calories. The association of air conditioners and television way have produced some pale, flabby, tired children who have gained excess weight during the warm weather.

These authors make several specific recommendations, including a plea for sustained vigorous outdoor activity, the use of water for quenching thirst, the control of high caloric drinks, cooling off periods before meals, and a continuation of all the usual measures for developing good eating habits.

Recent Nutrition Books

The Rockefeller Report on Education: The Pursuit of Excellence. Education and the Future of America. Special Studies Report V, Rockefeller Brothers Fund. Published by Doubleday & Company, Inc., Garden City, New York. Price $.75.

The World Health Organization: Its Global Battle Against Disease. Albert Deutsch. Public Affairs Pamphlets, 22 East 38th St., New York 16, N. Y. Pp. 20. Price $.25.

Food Guide and Quantity Control for Young Children, By the Community Council of Greater New York (foreword by Benjamin Spock, M.D.). Published by Harper & Brothers, New York. Pp. 177. Price $4.95.

Manual for Nutrition Surveys. Interdepartmental Committee on Nutrition for National Defense. U. S. Government Printing Office. Pp. 160. Price $1.50.

Food for Fitness. A Daily Food Guide. Leaflet no. 424, U. S. Department of Agriculture. U. S. Government Printing Office. Price: $.05 each; $3.75 per 100 copies.

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